

CLAIMS

1. A method of enumerating the number of CD 4 cells in a cell sample, the method comprising the steps of:
 - a) identifying the total CD 45 expressing population as a reference population for measuring CD 4 lymphocytes;
 - b) determining the percentage of CD 4 lymphocytes as a function of the total CD 45 reference population identified in step (a);
 - c) determining the number of CD 45 cells per volume of blood; and
 - d) calculating the number of CD 4 cells in the sample by multiplying the percentage of CD 4 cells, obtained in step (b), by the CD 45 count obtained in step (c).
2. A method according to claim 1, wherein the number of CD45 cells per volume of blood is determined by either a single platform method in which known numbers of beads are added to the sample and the beads and cells are counted simultaneously to obtain the absolute cell count, or by a dual platform method using a white blood cell count obtained from a haematology analyzer.
3. A method according to either of claims 1 or 2, wherein a combination of CD 45 and CD 4 antibodies is used to identify a five-part lymphocyte sub-set differential by an immunological method, comprising:
 - a) firstly, identifying total CD45 positive events, and subsequently defining the white blood cell differential using only CD4 and side scatter parameters, including an absolute lymphocyte count, an absolute monocyte count, an absolute granulocyte count, an absolute eosinophil count, and a CD 4 count, the method additionally comprising one or more of the following steps:
 - b) calculating the number of lymphocytes in the sample by relating the total proportion or total of the sums of the percentage of high density CD4++ bright cells and low side scatter cells, and the CD4- (negative) low side scatter cells, a component of which is CD4 positive lymphocytes, to the white blood cell population in the sample, thereby to establish the population of lymphocytes in the sample;
 - c) calculating the number of monocytes in the sample by relating the proportion or percentage of dim to moderate density CD4+ cells and medium side scatter cells to the white blood cell population in the sample, thereby to establish the population of monocytes in the sample;

- d) calculating the number of granulocytes in the sample by relating the proportion or percentage of negative to dim low density or autofluorescence CD4 negative cells and high side scatter cells to the white blood cell population in the sample, thereby to establish the population of granulocytes in the sample; and
 - e) calculating the number of eosinophils in the sample by relating the proportion or percentage of dim low density CD4+ cells and very high side scatter cells to the white blood cell population in the sample, thereby to establish the population of eosinophils in the sample.
- 4. A method according to claim 3, which further includes the step of calculating the number of basophils in the sample by relating the proportion or percentage of dim low density CD4+ cells and low side scatter cells to the white blood cell population in the sample.
 - 5. A method according to any one of claims 1 to 4, wherein the sample is whole unlysed blood, unfractionated, fractionated or lysed whole blood.
 - 6. A kit including CD4 and CD45 antibodies for use in enumerating the number of CD4 cells in a sample.
 - 7. A kit according to claim 6, which further includes instructions for performing the method of any one of claims 1 to 5.
 - 8. A kit according to claim 6, which further includes one or more reagents selected from the group consisting of a red cell lysating agent, a stabilizer, a fixative, control cells, media and bead reagents.
 - 9. A machine readable medium comprising instructions, which when executed by a machine, cause the machine to perform the method steps of any one of claims 1 to 5.
 - 10. A machine readable medium according to claim 9, which is configured for use in conjunction with a flow cytometer and/or haematology analyser.
 - 11. A machine readable medium according to either of claims 9 or 10, which includes instructions for performing analysis methods selected from the group consisting of impedance, light scatter, fluorescence and precision volume counting.

12. A method of monitoring the immune status of a patient with HIV or other immune deficiency condition or disease, the method including the step of enumerating the number of CD4+ lymphocytes or CD4+ T cells with reference to the total white blood cells in a cell sample from the patient by a method according to any one of claims 1 to 5.
13. A method according to claim 12, wherein the patient's immune status is determined or monitored to determine the patient's response to antiretroviral treatment.